Assessing the Effects of Pest Management on Nontarget Arthropods: The Influence of Plot Size and Isolation

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ABSTRACT Evaluations of field research on the nontarget effects of pest management, particularly the production of transgenic crops with insecticidal properties, suggest the methods used are sometimes unlikely to detect real differences among treatments. Among potential problems, abundance estimates may be scale-dependent for many arthropods, which move among experimental plots and between experimental plots and the surrounding environment. Insecticide-disturbed plots of field corn in a range of sizes in 2003 (0.03–0.53 ha; 18–72 m wide) and 2004 (0.01–0.13 ha; 9–36 m wide) were used for testing effects of scale on nontarget arthropod abundance. Possible effects of artificially isolating plots by removal of vegetation around plot borders were also studied in 2003. Community and taxon-based analyses showed abundance of foliar (aboveground) arthropods depended on plot size and isolation. While abundance of foliar arthropods was generally greater in smaller plots, isolation treatments suggested some taxa may have been either repelled or attracted to isolated plots. Levels of some epigeal (ground-dwelling) taxa were also size or isolation-dependent, but community-based analysis did not indicate a strong collective response to treatments. Recommendation of a practical but rigorous minimum plot size for nontarget studies may not be appropriate because responses to plot size varied among taxa. However, because arthropod movement into and out of plots can reduce differences between treatments, results suggest the use of small plots (width <9 m) for nontarget studies on transgenic crops generally should be avoided. Similarly, the taxon-specific effects of isolating plots should be considered when planning studies or interpreting results.

KEY WORDS experimental design, spatial scale, isolation, *Bacillus thuringiensis* (Berliner), principal response curves

IN AGRICULTURAL SYSTEMS, NONTARGET ARTHROPODS include all species other than those which pest management actions are intended to suppress. Some nontarget species are incidental or have little apparent significance to crop production, but many have valuable roles as decomposers, pollinators, predators, or parasitoids. Unintended harm to nontarget species may come from cultural practices (Thorbek and Bilde 2004), biological pest control (Simberloff and Stiling 1996, Louda et al. 2003), and pesticide use. The continuous expression of insecticidal proteins found in some transgenic crop varieties has also necessitated direct evaluations of their potential effects on nontarget arthropods.

While standardized laboratory tests provide invaluable information and guidance in assessing possible unintended effects of pest management, field trials represent the most realistic method for evaluating nontarget effects. Such tests simulate the environment in which pest management actions will be employed, providing normal climatic and agronomic conditions and allowing the complex interactions of all species present in an agricultural system. However, expenses including the costs of land and personnel often demand that field trials be conducted on a scale much smaller than the fields in which crops are typically produced. For tests of transgenic varieties under development, limitations on the availability of seed or area permitted for planting may also exist.

There may be additional legitimate constraints for compromising with regard to scale, but field research has shown that plot size influences assessments of arthropod density, damage, and distribution (Cantelo 1986, Jepson and Thacker 1990, Brown and Lightner 1997). Among several mechanisms that may cause such scale-dependence (Englund and Cooper 2003), movement of arthropods across plot boundaries is

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perhaps the most basic. The short distance from the center to the edge in smaller plots increases the likelihood that an individual will move across a plot border, whereas the relatively high perimeter-to-area ratio provides more opportunities for such movement by individuals within a population (Kareiva 1985, Sutcliffe et al. 1997, Englund and Cooper 2003). High per capita rates of immigration and emigration in small plots should generally reduce differences between plots; thus, the recovery time of arthropod populations after a disturbance is positively related to plot size (Duffield and Aebischer 1994). Even in groups for which recovery is not directly based on recolonization of disturbed areas, scale dependence may exist (Duffield and Aebischer 1994, Longley et al. 1997).

Isolation of experimental plots can be used to reduce movement of arthropods across plot boundaries and may be a condition of experimental use permits for transgenic crop varieties (to prevent cross-pollination). Whether plots are isolated by plantings of an alternate crop or separated by bare ground, the modification of plot borders and surrounding habitat can have unintended effects on levels of nontarget arthropods, which may be either attracted to or repelled by such borders (Duelli et al. 1990, Harwood et al. 1994, Lövei et al. 1998).

For testing nontarget effects of pesticides, which often have serious negative effects on arthropod communities, the influence of plot size or isolation is likely subtle. That is, any adverse nontarget effects should still be detectable, although the duration of such effects may differ. However, the potential nontarget effects of current transgenic crops with insecticidal properties [i.e., those expressing one or more *Bacillus thuringiensis* (Berliner) toxins] are expected to be relatively small (Wold et al. 2001). In this case, even minor problems with experimental design could greatly reduce the likelihood that effects on nontarget groups, adverse or beneficial, will be detected (EPA 2002). Even with potential for problems caused by using plots, not fields, as experimental units, some compromise with regard to scale may be needed as a practical measure. To determine how plot size and isolation may influence assessments of the impact of pest management on nontarget arthropods, season-long samples of a variety of nontarget taxa were collected in plots of field corn with and without applications of insecticides. Complementary analyses on the nontarget insect community and specific taxa were then used to examine (1) possible equivalence within a range of plot sizes and (2) effects of isolating experimental plots.

Materials and Methods

In 2003 and 2004, plots of field corn in which treatments varied with regard to insecticide use, plot size and border type were sampled once before and twice after each of three insecticidal disturbances of the arthropod community. Insecticide applications were intended to simulate the management of common corn pests but were based only on the likely timing of control measures for specific pests, not on their population levels. Because insecticide applications were made to plots covering only a small fraction (<13%) of a production-scale field, nontarget arthropods were expected to enter disturbed plots from insecticide-free areas. The time-series sampling of nontarget taxa was intended to show the recovery of nontarget arthropods after insecticidal disturbances, with analysis focused on how trajectories of the nontarget insect community and specific taxa differed between treatments. While only conventional (i.e., nontransgenic) corn hybrids were included, the experimental design employed has direct implications for field tests of transgenic crops with insecticidal properties; any effects of plot size and isolation on insecticide-managed areas, commonly used as positive controls in transgenic field trials, should impact comparative assessments of nontarget effects from transgenic crop production.

Table 1. Description of treatments assigned to field plots, 2003–2004

Year	Insecticides applied[a]	Plot size	Plot area	Plot border
2003	Yes	18 by 18 m	0.03 ha	Insecticide-free corn
	Yes	36 by 36 m	0.13 ha	Insecticide-free corn
	Yes	72 by 72 m	0.52 ha	Insecticide-free corn
	No	36 by 36 m	0.13 ha	Insecticide-free corn
	Yes	18 by 18 m	0.03 ha	6 m bare soil
2004	Yes	9 by 9 m	0.01 ha	Insecticide-free corn
	Yes	18 by 18 m	0.03 ha	Insecticide-free corn
	Yes	36 by 36 m	0.13 ha	Insecticide-free corn

[a] In 2003, applications were made to two-leaf (permethrin liquid, 0.22 kg A.I./ha), seven-leaf (permethrin granules, 0.22 kg A.I./ha), and silking stage corn (lambda-cyhalothrin, 0.03 kg A.I./ha). In 2004, an application was made at planting (tefluthrin, 0.18 kg A.I./ha), with permethrin granule and lambda-cyhalothrin applications made one growth stage later than in 2003.

2003. Hybrid corn (111 d maturity) was planted in a 24-ha field 8 km northwest of Slater, IA, where soybeans had been grown the preceding year. On corn emergence, plots were marked and assigned to one of five treatments (Table 1). The size of the field permitted four replications of all treatments with at least 45 m between any two plots and 40 m between any plot and the field border (Fig. 1).

After plots were established, two transects were marked with each transect beginning outside the plot border and intersecting the other at the center of the plot. Each plot contained one transect running north or south of the plot center and one transect running east or west, with combinations of compass directions assigned to an entire replicate. The number and location of points along transects depended on the size of the plot, with the 72 m/side plots containing nine points (3, 4.5, 9, 18, and 36 m north or south of plot borders; 3, 4.5, 9, 18 m east or west). With one fewer point along each transect, 36 and 18 m/side plots contained seven or five points, respectively. All plots had additional points along each transect at 3 and 6 m outside of the plot borders.

Pitfall traps were established at each point along the transects. For each trap, a bulb planter was used to

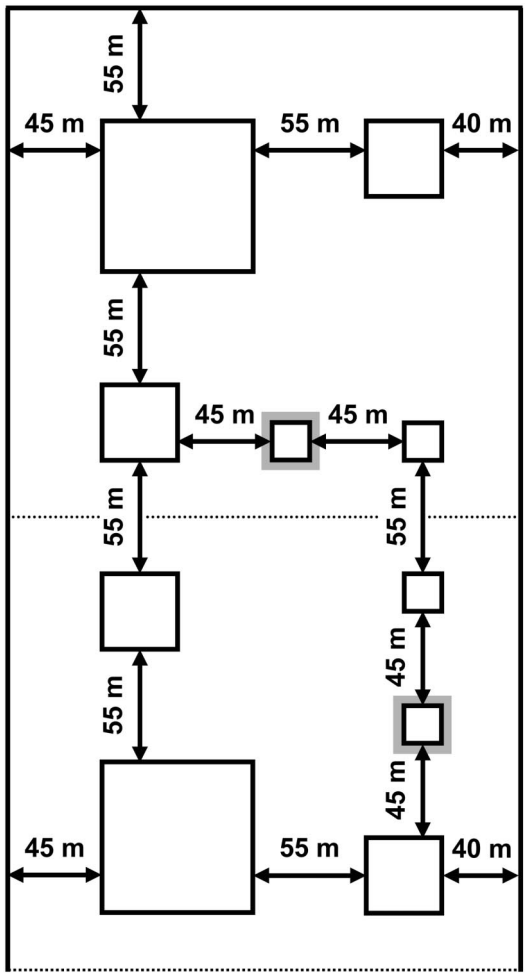

Fig. 1. Field layout for plot size and isolation tests in 2003. Dashed lines indicate boundaries between replicates, and specified dimensions refer to distances between plots or between a plot and a field border. Plot sizes are 18 by 18, 36 by 36, and 72 by 72 m. Shaded areas indicate removal of vegetation surrounding a plot. Diagram is approximately to scale, but only two of four replicates are shown.

remove a cylinder of soil and two nested clear plastic cups (414 ml, TP12; Solo Cup Co., Urbana, IL) were placed in the hole. The lower cup had holes drilled in the base to allow drainage in the event of rain, whereas the upper cup was filled with soil to retain the shape of the pitfall trap when not in use. The surrounding soil was manually leveled flush with the rim of the cup, and a cover made from two plastic plates (26.0 cm, PS15W; Solo Cup Co.) was placed over the trap to protect from rainfall or vertebrate animals. The two plates were held together by three bolts fastened to the plates by washers and nuts, with the bolts pushed into the soil to hold the plates with 2–3 cm between the plates and the ground. Just before corn reached the two-leaf (V2) stage of development (Ritchie et al. 1997), the soil-filled cup was removed and replaced by a cup partially filled with antifreeze (ethylene glycol-based) to collect and preserve epigeal (ground-dwelling) arthropods. After 24 h, the cups with antifreeze were collected, and the soil-filled cups and plate covers were replaced. When corn plants reached V2 stage, an insecticide application (Pounce 3.2 EC, permethrin, 0.22 kg A.I./ha, FMC Agricultural Products, Philadelphia, PA) was made to simulate chemical control of cutworm larvae (Lepidoptera: Noctuidae). Pitfall traps were subsequently used to sample epigeal arthropods 2 and 7 d after the V2 permethrin was applied.

As corn approached the seven-leaf stage (V7), pitfall traps were again used to sample the epigeal arthropod fauna. Yellow sticky traps and visual counts were also used to estimate the abundance of foliar (aboveground) arthropods at each transect point. One adhesive side of each sticky card (7.6 by 12.7 cm, Stiky Strips; Olson Products, Medina, OH) was exposed, and the card was secured with clothespins to a bamboo pole pushed into the ground. The position of each card was at the height of the corn canopy with the exposed adhesive facing toward the center of the plot. After 24 h, the cards were removed and placed into clear plastic bags. Visual counts of specific arthropod taxa were made on 10 plants per transect point, with attempts made to count nonconsecutive plants within 1.5 m of the transect point. When corn plants reached the V7 stage, a granular insecticide application (Pounce 1.5 G, permethrin, 0.22 kg A.I./ha) was made to simulate chemical control of the first generation of the European corn borer [*Ostrinia nubilalis* (Hübner); Lepidoptera: Crambidae]. Pitfall, sticky card, and visual sampling were repeated at 2 and 7 d after the granules were applied. The process of sampling, applying an insecticide, and collecting two postapplication samples was repeated again when corn plants approached silking stage (R1). At this point, corn plants exceeded the height of the bamboo poles, so sticky cards were placed at the height of the lowest developing corn ear. This final insecticide application (Warrior, lambda-cyhalothrin, 0.03 kg A.I./ha, Syngenta Crop Protection, Greensboro, NC) was made to simulate an insecticide application to suppress second-generation European corn borers. A summary of the arthropod sampling, including methods, timing, nontarget groups, and life stages counted is shown in Table 2.

After collection, samples from all pitfall traps were briefly stored in a temperature-controlled room at 4°C. As soon as possible, the antifreeze was removed, and all collected arthropods were placed into a 70% ethanol solution and returned to storage until the samples could be sorted. For identification, samples were separated from alcohol and positioned onto filter paper using a vacuum system. Samples on the filter paper were identified under a dissecting microscope. Absolute counts of all groups were made except for collembolans, which were estimated by counting the number on 10% of the area of filter paper and extrapolating (multiplying by 10). Sticky cards were stored in a freezer (−16°C) until they could be examined using a dissecting microscope.

Table 2. Groups of nontarget arthropods sampled, 2003–2004

Sampling method	Sampling periods[a]	Nontarget group	Life stages
Pitfall traps	2003:V2,V7,R1	Lycosidae (Araneae)	Nymphs, adults
	2004:PT,V8,R2	Other Araneae	Nymphs, adults
		Opiliones	Nymphs, adults
		Chilopoda	Larvae, adults
		Collembola (elongate)	Nymphs, adults
		Collembola (globular)	Nymphs, adults
		Carabidae	Larvae
		Harpalus spp.	Adults
		Pterostichus + *Poecilus* spp.[b]	Adults
		Staphylinidae	Adults
		Staphylinidae	Larvae
Yellow sticky cards	2003:V7, R1	*Orius insidiosus* (Say)	Adults
	2004:V8, R2	Cicadellidae	Nymphs, adults
		Aphididae	Nymphs, adults
		Thysanoptera	Larvae, adults
		Dolichopodidae	Adults
		Syrphidae	Adults
		Mymaridae	Adults
		Trichogramma sp.	Adults
		Macrocentrus cingulum Reinhard[c]	Adults
Visual counts	2003:V7, R1	*Orius insidiosus* (Say)[c]	Adults
	2004:V8, R2	Chrysopidae	Eggs
		Chrysopidae	Larvae
		Coccinellidae	Larvae
		Coleomegilla maculata DeGeer	Adults
		Harmonia axyridis (Pallas)	Adults
		Coccinella septempunctata L.	Adults

[a] Sampling period abbreviations: PT, planting time. Other periods correspond to (V) vegetative and (R) reproductive stages of corn described by Ritchie et al. (1997).
[b] Combined genera abbreviated as *Pterostichus* spp. in figures.
[c] Only counted or sampled with this method in 2004.

2004. Hybrid corn (107 d maturity) was planted in the same field as in 2003. Although the overall experimental design used the prior year seemed successful, preliminary analyses of 2003 data indicated areas with potential for improvement. First, it seemed the earliest insecticide application in 2003 did not adequately disturb the epigeal nontarget community. Consequently, the first insecticide application in 2004 (Force 3G, tefluthrin, 0.18 kg A.I./ha, Syngenta Crop Protection, Greensboro, NC) was made at planting time to simulate insecticidal suppression of corn rootworm larvae (Coleoptera: Chrysomelidae). This change to the insecticidal disturbance schedule was also more realistic in terms of common corn pests in Iowa, particularly considering the increased problems caused by *Diabrotica* spp. when corn is grown in consecutive years at the same location (Nelson et al. 1994). Also, it seemed that analyses of nontarget groups responding most strongly to treatment effects (as indicated by initial community-level tests) might have insufficient power to detect treatment differences if analyzed separately at the taxon level. In particular, data for some taxa seemed to have high variability relative to mean abundance (i.e., coefficient of variation = $[\sigma/\mu] \times 100$; Sokal and Rohlf 1995). This was addressed in two ways. First, to raise mean abundance of taxa collected by trapping, all pitfall and sticky traps were left in the field for 72 h (increased from 24 h). Second, to overcome naturally high variation, the number of replicates per treatment was increased from 4 to 10.

The marked increase in replication and the need to isolate plots from one another and field borders ($\geq$35 m) required reducing the number of treatments and size of plots used in 2004 (Table 1). Similarly, the number and location of points along transects were changed. All plots contained five points, but the location of points along each of the two transects were proportional to plot size (one-sixth, one-third, or one-half the length of the plot; e.g., points 3, 6, and 9 m inside the borders of 18 m/side plot). Modifications to transects were made to reduce potential bias of sampling effort in favor of larger plots and areas near plot borders.

Data Analysis. To detect effects of insecticide applications, plot size, and isolation on the community of nontarget arthropods, time-series data were collected on >20 distinct groups. As an alternative to analyzing all taxa independently with a series of repeated-measures analyses of variance [RM-analysis of variance (ANOVA)], a multivariate approach called principal response curves (PRCs) was used. PRCs provide a means to visualize and test for the response of a community to environmental stress (Frampton et al. 2000, van den Brink et al. 2000, Naranjo et al. 2003). van den Brink and ter Braak (1999) provide details regarding the development of PRCs and the underlying calculations.

Analysis using PRCs is similar to the more common principal components analysis, where latent variables (axes) are extracted from a matrix of species abundance data. In PRCs, values of one or more latent variables are then subjected to weighted least-squares regression on treatment and time variables to produce canonical coefficients (c_{dt}). Weighting in the regres-

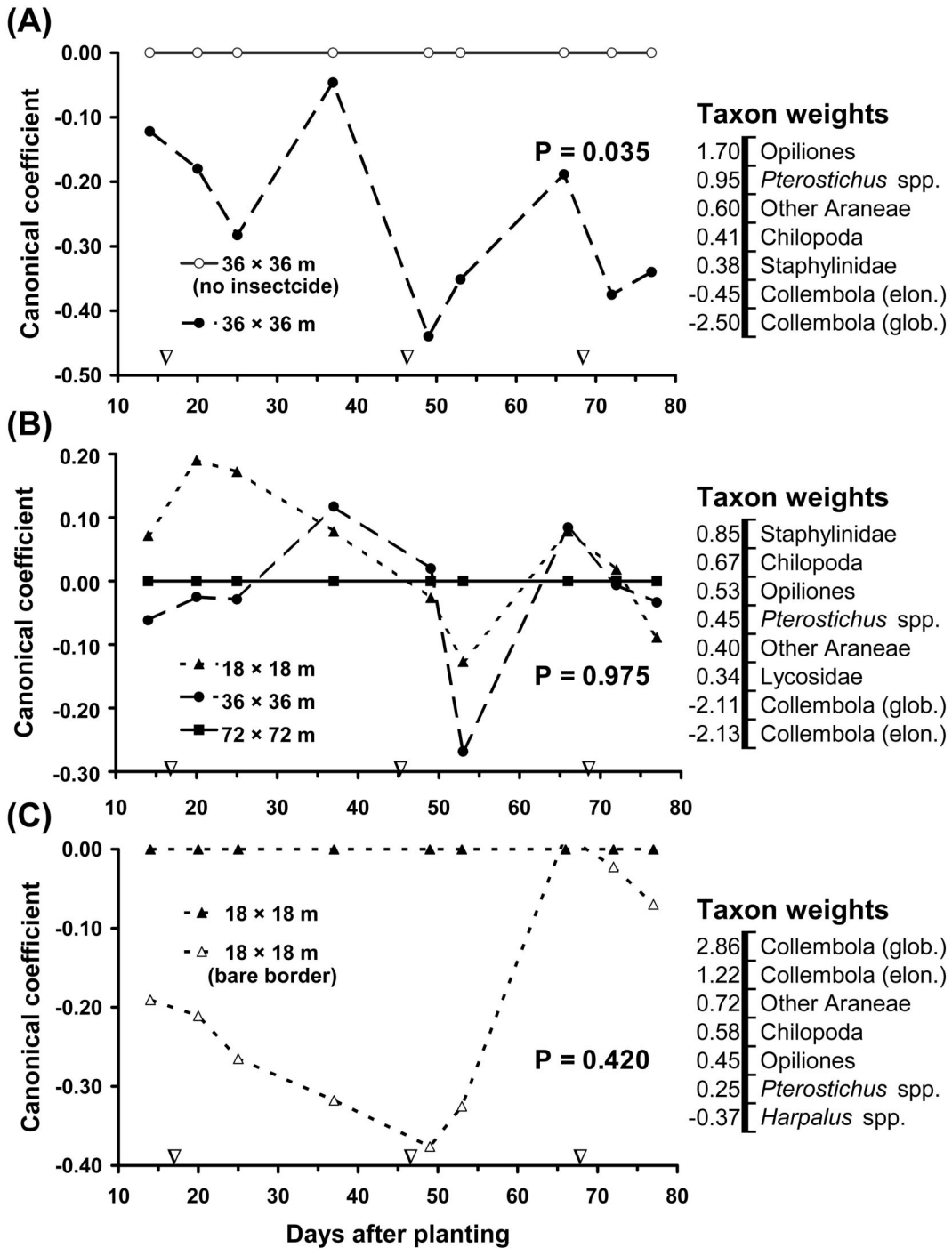

Fig. 2. PRC diagrams showing the community responses (c_{dt}) of epigeal arthropods to (A) insecticide applications, (B) plot size, and (C) border type during 2003. *P* values express the significance level for the relationship between the first latent variable (axis) and the time-dependent treatment effects. Taxon weights are shown only for groups with $|b_k| \geq 0.25$. Taxa with $b_k \geq 0.5$ generally follow the displayed community pattern, whereas those with $b_k \leq -0.5$ exhibit a pattern contrary to the community response. ▽, timing of insecticide applications.

sion model is based on the response of each taxon relative to the abundance of the taxon in a designated control. Thus, community responses are expressed as deviations from a control community. Coefficients (typically from the first axis) are plotted on PRC diagrams, line plots of the community response to

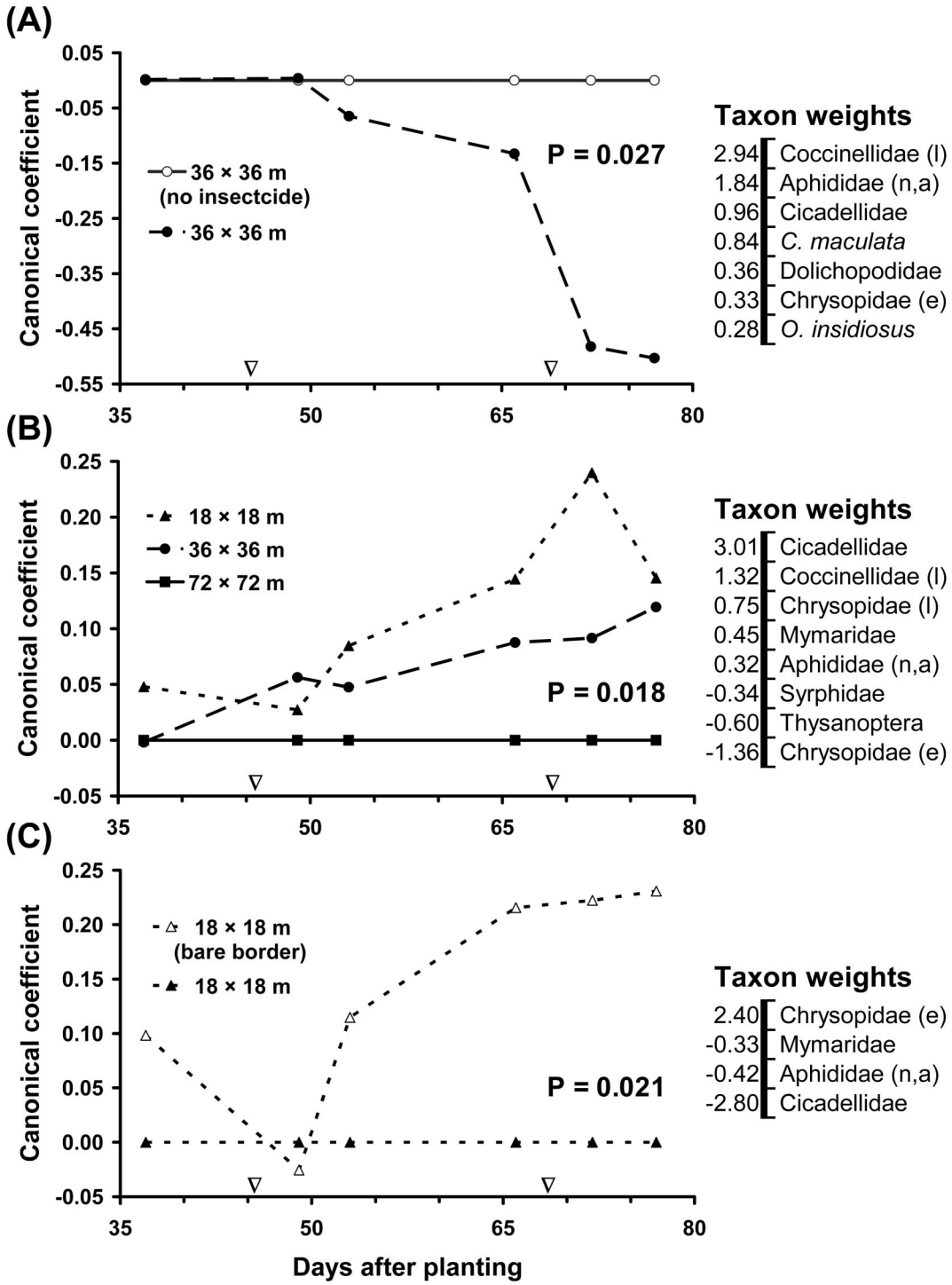

Fig. 3. PRC diagrams showing the community responses (c_{dt}) of foliar arthropods to (A) insecticide applications, (B) plot size, and (C) border type during 2003. *P* values express the significance level for the relationship between the first latent variable (axis) and the time-dependent treatment effects. Taxon weights are shown only for groups with $|b_k| \geq 0.25$. Taxa with $b_k \geq 0.5$ generally follow the displayed community pattern, whereas those with $b_k \leq -0.5$ exhibit a pattern contrary to the community response. ▽, timing of insecticide applications.

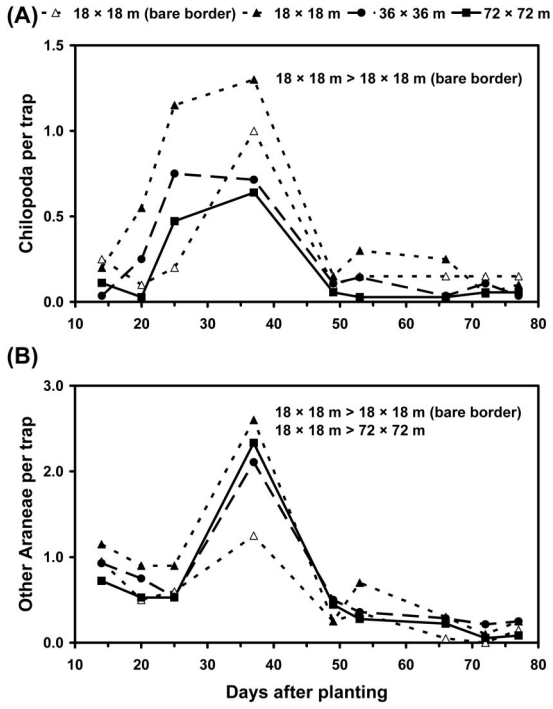

Fig. 4. Mean number of (A) centipedes and (B) nonlycosid spiders per pitfall trap displayed by treatment and time, 2003. Means are shown without error estimates to allow clearer discrimination of treatments. Treatment differences detected using RM-ANOVA are shown by >.

treatments over time. By convention, a treatment is designated as the control and plotted as a straight line to aid in visual separation of treatments. PRCs generate a second plot of species weights (b_k) that are used to indicate which species follow the plotted community pattern. Taxa with large, positive weights ($b_k \geq 0.50$) generally follow the plotted community response in a PRC diagram, whereas those with large, negative weights ($b_k \leq -0.50$) exhibit trends contrary to the plotted community response to treatments over time.

Quantitative tests of whether a given PRC diagram displays significant variance because of treatment are provided by Monte Carlo permutations. Distribution-free *F*-type tests are produced by (1) calculating a test statistic, F_0, which measures how much of the variance caused by treatment and time effects are explained by the plotted community response, (2) generating K new data sets by randomly permuting (shuffling) samples between specific groups (e.g., treatments), (3) calculating test statistics, $F_1 - F_k$, for each data set, and (4) determining significance level by placing F_0 within the group of $K + 1$ sets (ter Braak and Šmilauer 2002). For example, with 999 permutations, a test statistic (F_0) with a rank of 40 would have a significance level of 0.040 (40/[999 + 1]).

PRC diagrams and corresponding Monte Carlo tests were made to test for effects of insecticide use, plot size, and border vegetation on the epigeal and foliar nontarget communities using CANOCO software (ter Braak and Šmilauer 2002). Data were formatted to reflect the mean number of each nontarget group per transect point within a plot (i.e., per 10 plant visual count, pitfall or sticky trap, excluding traps outside plot borders) to allow comparison of plots containing different numbers of points, and subsequently $\log_{10}(x + 1)$-transformed. Monte Carlo tests were configured to allow permutation between treatments but not between dates. Only the treatments appropriate to an effect were included in a specific test. For example, the test for the effect of insecticide use in 2003 compared only plot of equal size (36 by 36 m), with and without insecticides. Tests for groups sampled with pitfall traps and for other nontarget taxa were conducted separately because visual counts and sticky cards were only used in the last six of nine sampling periods in each year.

Additional statistical analyses were conducted using SAS software (SAS Institute 1999). Repeated-measures ANOVA were performed on nontarget groups likely responsible for community responses to plot size and border treatments as indicated by PRC analysis. Groups with $|b_k| \geq 0.40$ from PRC analyses were tested, creating a slightly more inclusive set of subjects than by using $|b_k| \geq 0.50$ as a selection criterion. These analyses were performed to (1) allow effects on certain taxa to be viewed and tested directly and (2) provide a more conventional method of analysis for time-series abundance data. For each taxon, a repeated-measures model (PROC MIXED) tested whether treatment (between-subject effect), time (within-subject effect) and an interaction of treatment and time had a significant relationship to the $\log_{10}(x + 1)$-transformed mean number of individuals captured per transect point inside plots. To test for effects of plot size and border treatments, but not for the direct effects of insecticide treatments (which should be detectable using the PRC analysis), data from insecticide-free control plots were excluded in all ANOVA. Degrees of freedom for *F*-tests of model effects were determined using the Kenward-Roger method to limit the type I error rate. Repeated samples of a plot were related using a first-order autoregressive (AR[1]) or heterogeneous first-order autoregressive (ARH[1]) covariance structure. When the RM-ANOVA results indicated a significant treatment effect, post-ANOVA analysis used paired comparisons (CONTRAST statements) to test for effects of border vegetation and plot size. Tests for a border effect compared equally sized (18 by 18 m) treatments immediately surrounded by insecticide-free corn or a 6-m band of soil with all vegetation manually removed. Tests for plot size effects used the largest plot size as a control, with paired comparisons of each of the two smaller plot sizes against the largest. If results indicated a significant treatment effect and a treatment × time interaction, paired comparisons (CONTRAST statements) were made to test for effects of border vegetation and plot size within each sample date.

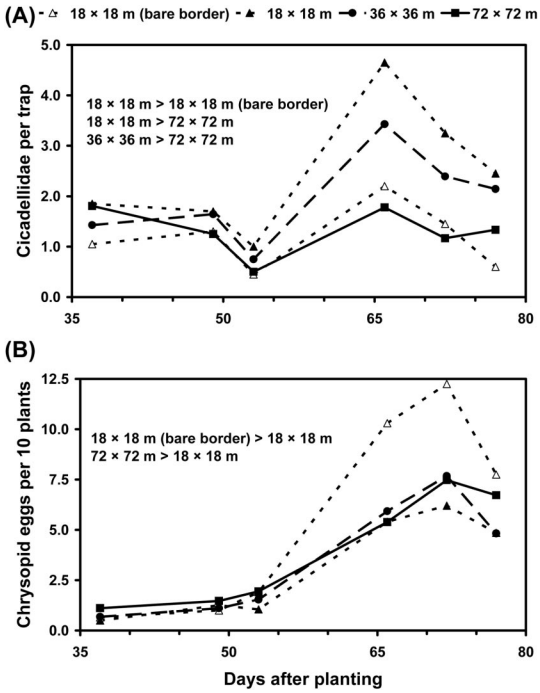

Fig. 5. Mean number of (A) leafhoppers and (B) lacewing eggs per sampling unit (sticky trap or 10 plant visual count) displayed by treatment and time, 2003. Means are shown without error estimates to allow clearer discrimination of treatments. Treatment differences detected using RM-ANOVA are shown by >.

Results

2003. Analysis of community data using permutation tests for PRCs indicated a significant negative effect of insecticide applications on epigeal taxa ($P = 0.035$, Fig. 2A), but this disturbance failed to produce a clear pattern of community-level effects when only plot size was considered ($P = 0.975$, Fig. 2B). Similarly, time-dependent treatment effects were not found for the epigeal community when only the effect of plot border was analyzed ($P = 0.420$, Fig. 2C). For the foliar arthropod community, effects of insecticides ($P = 0.027$), plot size ($P = 0.018$), and plot border ($P = 0.021$) were all apparent using principal response curves analysis (Fig. 3A–C).

Repeated-measures analyses of taxa with absolute weights ($|b_k|$) ≥ 0.40 from PRC analyses of plot size or border type indicated significant treatment effects and no treatment × time interactions for four of the taxa tested. Among the epigeal taxa, no differences were detected for the groups with the largest $|b_k|$, but plot type effects for nonlycosid spiders (other Araneae; $F_{3,28.4} = 3.55$, $P = 0.027$) and centipedes (Chilopoda; $F_{3,25.7} = 4.01$, $P = 0.018$) were detected (Fig. 4). Treatment effects were also found for green lacewing eggs (Chrysopidae; $F_{3,17.7} = 4.63$, $P = 0.015$) and leafhoppers (Cicadellidae; $F_{3,16.9} = 8.69$, $P = 0.001$), the taxa with the largest PRC weights for plot size and border effects in the foliar community (Fig. 5). Significant results from the three planned comparisons are displayed for each group in Figs. 4 and 5.

2004. As in 2003, PRC analysis of the epigeal arthropod community in plots that received insecticide applications did not show effects of plot size ($P = 0.480$, Fig. 6A), but community-level effects of plot size on foliar taxa were indicated ($P = 0.016$, Fig. 6B). Repeated-measures ANOVA on epigeal taxa weighted (b_k) ≥ 0.40 in PRC analyses showed only centipedes responding to plot size ($F_{2,47.2} = 5.75$, $P = 0.006$; Fig. 7). Among groups counted using sticky traps or visual samples, both treatment (i.e., plot size) effects and treatment × time interactions were found for ladybeetle larvae (Coccinellidae; treatment $F_{2,51.6} = 4.90$, $P = 0.011$; interaction $F_{10,137} = 2.40$, $P = 0.012$), long-legged flies (Dolichopodidae; $F_{2,61.7} = 5.42$, $P = 0.007$; $F_{10,136} = 1.98$, $P = 0.040$), and insidious flower bugs (*O. insidiosus*; $F_{2,56.5} = 10.01$, $P < 0.001$; $F_{10,77.2} = 2.70$, $P = 0.007$). Consequently, analyses of plot size effects were made within each sampling period, with results of the two pairwise comparisons as indicated in Fig. 8.

Discussion

Results indicated that plot size and isolation influenced nontarget arthropod levels in insecticide-disturbed areas. These effects were clearest on foliar arthropods, for which community-level analyses using PRCs indicated effects of plot size (Figs. 3B and 6B) and border (Fig. 3C) treatments. Subsequent ANOVA on specific nontarget taxa supported the PRC results. Using the largest plot size in each year as a control for comparison with two smaller plot sizes, significant differences were found for five of the groups of foliar arthropods sampled (Figs. 5 and 8). Comparisons between plots which received insecticide applications with or without removal of surrounding vegetation showed a significant effect of isolation (Fig. 5) on the number of leafhoppers and lacewing eggs.

However, for epigeal arthropods in 2003 and 2004, disparities were found between the community- and taxon-level analyses. Although insecticides had a negative impact (Fig. 2A), community recovery did not seem to be related to the range of plot sizes tested (Figs. 2B and 6A) or to the isolation of plots (Fig. 2C). However, the lack of a consistent pattern at the community level did not preclude the presence of taxon-level effects of plot size (nonlycosid spiders, Fig. 4B; centipedes, Fig. 7) or isolation (centipedes and nonlycosid spiders, Fig. 4A and B). At least two factors may explain the differences between results at the community and taxon levels. First, the PRC analysis is more powerful when several included taxa show similar treatment responses. This did not seem to be the case for either the plot size or isolation effect. Second, the most frequent and abundant taxa (elongate and globular collembolans, or collectively, springtails) strongly influenced the PRC results as indicated by the large taxon weights (Figs. 2B and C and 6A). The low potential for recolonization of disturbed plots by springtails, which are likely less mobile than other taxa

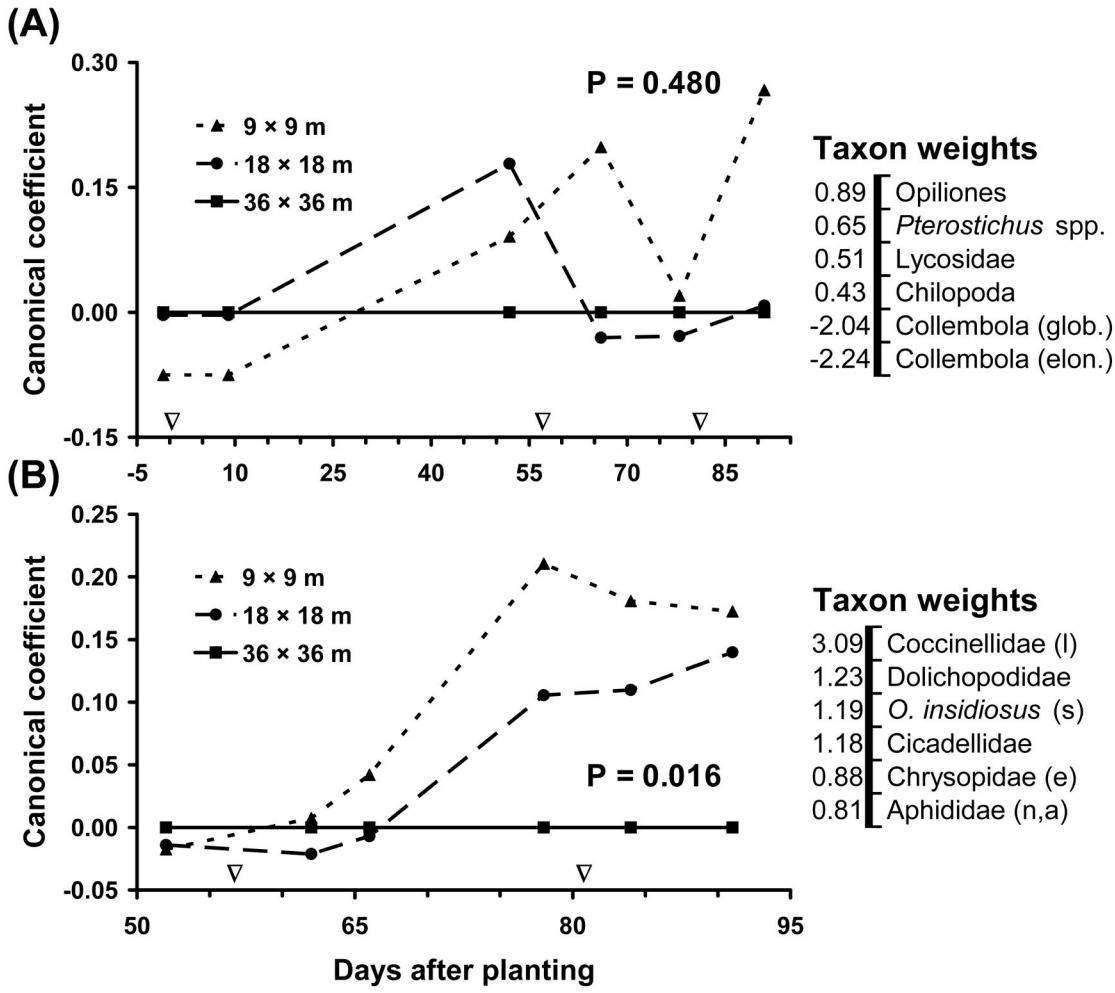

Fig. 6. PRC diagrams showing the community responses (c_{dt}) of (A) epigeal and (B) foliar arthropods to plot size during 2004. *P* values express the significance level for the relationship between the first latent variable (axis) and the time-dependent treatment effects. Taxon weights are shown only for groups with $b_k \geq 0.25$. Taxa with $b_k \geq 0.5$ generally follow the displayed community pattern, whereas those with $b_k \leq -0.5$ exhibit a pattern contrary to the community response. ▽, timing of insecticide applications. Note the differences in scale of x-axis between A and B.

collected, may have biased the community analysis against finding plot size and isolation effects.

Although precise responses to plot size differed among taxa (as would be expected based on variation in mobility and behavior; Jepson 2002), the expected trend of higher populations in smaller plots was apparent. Several other groups that did not show significant responses to plot size exhibited a logical ordering of mean abundances (smallest > intermediate > largest plot size), suggesting possible treatment differences that were not detectable with ANOVA (data not shown). This pattern is a result of greater movement of arthropods into small plots from surrounding areas of undisturbed corn; as previously noted, the relatively short distance from the border to the center of smaller plots also allows more rapid colonization by individuals, whereas the relatively high perimeter-to-area ratio promotes increased per capita exchange of individuals across plot borders (Kareiva 1985, Sutcliffe et al. 1997, Englund and Cooper 2003).

One unusual result was seen for green lacewing eggs in 2003 (Fig. 5B), which were more abundant in the largest (72 by 72 m) plots compared with the smallest (18 by 18 m) plot treatment; although not tested, all plots that received insecticide applications seemed to have fewer lacewing eggs than the insecticide-free controls. Egg mortality in response to pyrethroid use (Schuster and Stansly 2000) could result in inaccurate, high visual counts if dead lacewing eggs are counted along with recently deposited, viable eggs. If caused by residual toxicity, such an effect might be stronger in larger plots, in which more of the surrounding area is exposed to insecticides. Another hypothesis relates to a possible repellency response of adults to the insecticide treatments (Wiles and Jepson 1994, Umoru et al. 1996). If sublethal effects on adult lacewings

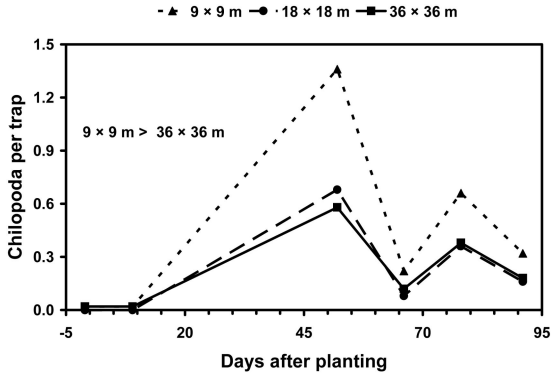

Fig. 7. Mean number of centipedes and per pitfall trap displayed by treatment and time, 2004. Means are shown without error estimates to allow clearer discrimination of treatments. Treatment differences detected using RM-ANOVA are shown by >.

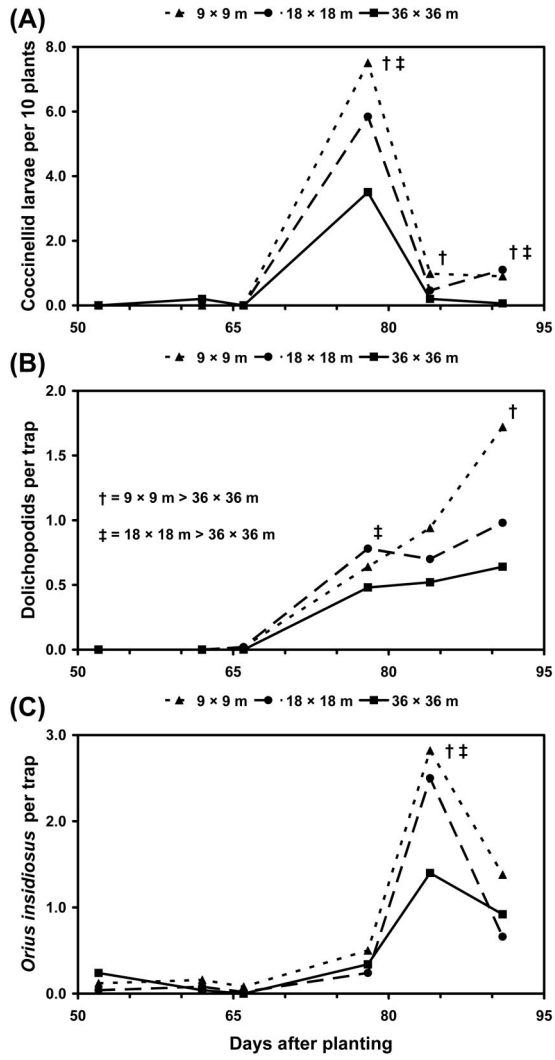

Fig. 8. Mean number of (A) coccinellid larvae, (B) dolichopodids, and (C) *O. insidiosus* per sampling unit (sticky trap or 10 plant visual count) displayed by treatment and time, 2004. Means are shown without error estimates to allow clearer discrimination of treatments. Treatment differences detected using one-way ANOVA for each date are indicated by † and ‡.

increase their tendency to move, they may be less likely to leave larger plots than smaller ones. Other nontarget studies show similar counterintuitive results for some natural enemy taxa in positive control treatments (Dively 2005, Lopez at al. 2005). Mechanisms other than those noted above, including reduced competition for resources, scavenging, and hormoligosis (i.e., hormesis; Morse 1998) all may produce such unexpected results.

The effects of border treatments on nontarget groups also differed. The 6-m soil border reduced the number of leafhoppers, centipedes, and nonlycosid spiders in plots (Figs. 4A and B and 5A), but greatly increased the abundance of lacewing eggs (Fig. 5B). The difference in type of effect (positive or negative) between these groups is consistent with previous research; whereas many arthropods seem to be repelled by bare ground (Duelli et al. 1990, Harwood et al. 1994, Lövei et al. 1998), some flying insects (particularly herbivores) are more attracted to crop plants surrounded by soil (Smith 1976a, b). This may be a result of greater visual apparency caused by the high contrast between soil and vegetation (Prokopy and Owens 1983). Previous research suggests that isolation of plots using other crops or noncrop vegetation would also be expected to influence movement into or out of plots for many species (Grez and Prado 2000, Collinge and Palmer 2002).

Differences in arthropod densities among the plot sizes tested (18-, 36-, and 72-m widths [2003]; 9, 18, and 36 m [2004]) suggest experiments with the smallest plot sizes (9 and 18 m) are not equivalent to those conducted in larger plots or field-sized replicates. At the same time, recommendation of an acceptable plot size is complicated because different taxa exhibit different responses to plot size. At a minimum, results support the conclusion that even preliminary nontarget studies (particularly for transgenic crops) should avoid the use of very small (3- and 5-m plot widths) common in the earliest publications on the nontarget effects of transgenic crops. Furthermore, because the size of commercial corn fields far exceeds that of the largest plots used in 2003 (0.53 ha) and 2004 (0.13 ha), effects of scale on nontarget arthropods may exist beyond the plot sizes tested. Similarly, the effects of isolating experimental plots should be considered when planning studies or interpreting results. Although plot borders also have taxon-specific effects, results of previous research and generalizations for some nontarget groups (e.g., based on type or speed of dispersal, mode of host, or prey location) can assist in deciding whether to use bordered plots and what type of border is most appropriate. Clearly, recommendation of larger plot sizes increases competition for limited funding, particularly with mounting evi-

dence suggesting increased replication is also needed for transgenic nontarget research (Bourget et al. 2002, Lopez et al. 2005, Naranjo 2005). However, both issues may simply be indications that field tests with transgenic crops will require either greater investment or innovations in experimental design and analysis to have adequate power to detect treatment differences.

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